Culture-Negative Intracerebral Abscesses in Children and Adolescents from *Streptococcus anginosus* Group Infection: A Case Series

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We report the use of 16S ribosomal RNA gene amplification and sequencing to diagnose culture-negative intracerebral abscesses in younger patients. These 3 cases demonstrate the optimal application of gene sequencing from direct specimens for patients with negative culture results compromised by antibacterial therapy but histories highly suggestive of acute bacterial infection.

Bacterial intracerebral abscess in children and adolescents is a serious condition often leading to permanent neurologic sequelae or death [1, 2]. Historically, anginosus group streptococci have contributed to most cases of intracerebral abscess in children resulting from hematogenous spread from a distant focus (e.g., congenital heart disease) or extension from a contiguous focus of infection (e.g., sinus, teeth, and middle ear) [1–4]. Although anginosus group streptococci are not considered fastidious, patients with life-threatening infection often receive antibacterial therapy prior to specimen collection, which can compromise bacterial growth and culture results. Twenty-four to 40% of all intracerebral abscesses produce negative culture results [2, 5, 6], due in large part to patients receiving antibacterial therapy [7].

We report the use of 16S rRNA gene sequencing from direct specimen to diagnose conditions in 3 patients with culture-negative intracerebral abscesses. These cases demonstrate the optimal application of gene sequencing from a direct specimen in critically ill patients with negative culture results, for whom clinical histories were highly suggestive of an acute bacterial infection, who received antimicrobial therapy prior to specimen collection, and who had positive Gram stain results from biopsy material.

**Case reports.** Patient 1 was a 6-year-old boy with Ebstein anomaly who presented with a 5-day history of bifrontal headache. One day prior to hospital admission, he complained of vomiting and syncope. He denied experiencing neck stiffness, photophobia, nasal congestion, cough, sore throat, or recent trauma. He had recurrent otitis media and patent foramen ovale with bidirectional shunting. Physical examination revealed that he was afebrile and had stable vital signs. His neck was supple, but fundoscopic examination revealed bilateral papilledema. He had dental caries. Cardiac examination results had not changed since baseline. Blood samples were obtained for culture, and treatment with ceftriaxone and vancomycin was initiated. Brain MRI demonstrated multiple ring-enhancing lesions consistent with septic emboli-producing parenchymal abscesses.
with septic emboli (figure 1). Transesophageal echocardiography revealed bidirectional patent foramen ovale without cardiac vegetations. Maxillofacial CT demonstrated right maxillary sinusitis. Six blood cultures produced negative results for bacterial pathogens. Intracerebral biopsy of an abscess demonstrated gram-positive cocci, but culture results from this specimen were negative. Amplification and sequencing of the 16S rRNA gene were performed from surgically obtained specimens using a modified protocol by Nikkari et al. [8]. *Streptococcus intermedius* was detected (100% identity; GenBank accession number AF169357). Antibiotic treatment was changed to meropenem, and the child underwent 13 weeks of therapy. The patient experienced residual impairment in behavior.

Patient 2 was a 21-year-old man experiencing sinusitis and bronchitis who presented with a 1-week history of fevers, fatigue, and neck pain. Lumbar puncture revealed cloudy CSF, with a WBC count of 4600 cells/µL (neutrophils, 77%; lymphocytes, 15%), a glucose level of 47 mg/dL, a protein level of 138 mg/dL, and no organisms detected by Gram stain. Blood and CSF culture results were negative for bacteria and fungi. Ceftriaxone treatment was initiated, and the patient underwent 14 days of therapy. He returned 2 weeks later with slurred speech. Head CT demonstrated multiple ring-enhancing lesions with hydrocephalus. He started receiving treatment including ceftriaxone, vancomycin, metronidazole, and corticosteroids, with resolution of symptoms. Seven days later, the patient developed a headache, photophobia, and hyperacusis. At that time, it was noted that histoplasma serologic test results were positive (mycelial antibody result, negative; yeast antibody titer, 1:32), and a lipid formulation of amphotericin B was added to his treatment regimen. The patient underwent a stereotactic-guided, right frontal craniotomy including removal of an abscess. Intra-operative material from the abscess demonstrated gram-positive cocci in chains, but bacterial and fungal culture results were negative. Transthoracic echocardiography and chest CT findings were not contributory. The patient continued to receive lipid-formulation amphotericin B treatment because histoplasmosis could not be definitively excluded. He completed a 6-week regimen of antibacterial therapy for treatment of worsening cerebral edema. Histopathologic test results of the intracerebral specimen revealed acute inflammation with early granulation tissue consistent with abscess formation, but no microorganisms were detected. Cultures of a tracheal aspirate specimen yielded a pure growth of *Streptococcus constellatus*. 16S rRNA gene amplification and sequencing detected *S. intermedius/constellatus* (99.8% identities; GenBank accession numbers AF04671 and AY277942) from 2 of 3 intracerebral specimens. The patient received ceftriaxone and rifampin treatment for 16 weeks. The patient experienced residual behavioral abnormalities.

**Discussion.** The 3 cases in this report illustrate the diagnostic value of broad-range gene amplification and sequencing from direct specimens for patients with culture-negative infections who have a high likelihood of an acute bacterial infection, received antibacterial therapy prior to specimen collection, and have biopsy material with positive Gram stain results. Anginosus group streptococci were the etiologic agents for these 3 cases of intracerebral abscess and could only be detected by 16S rRNA gene amplification and sequencing. Definitive microbial identification by 16S rRNA gene sequencing was available in real time for 2 of the 3 cases, enabling clinicians to tailor antibiotic therapy, discontinue vancomycin treatment, and avoid further diagnostic procedures.

Children and adolescents with bacterial intracerebral abscess are usually immunocompetent and present with community-acquired infection [1, 9]. Although most descriptions of intracerebral abscesses published elsewhere are all-inclusive (e.g., adults, children, and immunocompromised individuals), it appears that congenital cardiac anomalies, sinusitis, and otitis media, in decreasing order, are still the most common predis-
posing risk factors in immunocompetent children, with an observed increasing proportion associated with neurosurgical procedures and trauma [1, 5, 9]. Interestingly, reported incidences of intracerebral abscess are much higher among male patients than female patients, with ratios ranging from 1.3:1 to 3.0:1 [2, 5, 9]. All 3 patients in our study were male.

Anginosus group (“milleri group”) streptococci are the most common pathogens associated with bacterial intracerebral abscesses [1–4]. _S. intermedius_, _S. constellatus_ and _Streptococcus anginosus_ are the 3 distinct streptococcal species that constitute anginosus group streptococci. _S. anginosus_ is commonly associated with bacteremia and gastrointestinal and genitourinary abscesses [10–12]. _S. constellatus_ and _S. intermedius_ are 2 species that are closely related phylogenetically. _S. constellatus_ has been implicated in all types of deep abscesses, and _S. intermedius_ has been strongly associated with intracerebral abscesses [10–13]. Greater than 90% of anginosus group streptococci are susceptible to penicillin, with _S. intermedius_ having the lowest geometric mean penicillin MIC (at 0.037 μg/mL) [14].

The first patient we described with _S. intermedius_-associated intracerebral abscess had multiple known risk factors for the development of invasive CNS disease, including congenital heart disease, sinusitis, otitis media, and dental caries. Despite the prompt initiation of antibacterial therapy, this patient did not return to his baseline function. Permanent neurologic sequelae are a frequent complication of intracerebral abscesses in children [1]. The second case of _S. intermedius_ infection highlights the pathogenesis of intracranial abscesses in patients with concurrent sinusitis; viridans group streptococci are reportedly the most prevalent pathogens associated with this contiguous infection [5]. Although we acknowledge that histoplasmosis could not be definitively excluded, cases of brain abscesses from histoplasma are rare, and Gram stain of biopsy material demonstrated gram-positive cocci consistent with the diagnosis of _S. intermedius_ infection. Regarding the third described patient, we hypothesize that isolation of _S. constellatus_ in pure culture of a tracheal aspirate specimen in the presence of a lung abscess confirmed that _S. constellatus_ was the source of CNS disease via a hematogenous route. Despite the high association between _S. intermedius_ and intracerebral abscess, Clarridge et al. [10] evaluated 60 patients with abscess due to anginosus group streptococci and reported that _S. constellatus_ was more frequently recovered from pleuropulmonary sources than _S. intermedius_. To our knowledge, no series has been published describing a case of concurrent thoracic and intracerebral abscesses caused by anginosus group streptococci.

Specimen culture still remains the most sensitive method to detect invasive bacterial infection in the CNS, because amplification and sequencing of broad-range DNA targets are often limited by clinical sensitivity and bacterial contamination [15]. Clinical sensitivity is greatly improved when this molecular technique is applied to patients whose culture results remain negative, but whose histopathologic and Gram stain results of specimens are suggestive of an acute bacterial infection. As we have shown, in select clinical contexts, the application of 16S rRNA gene amplification and sequencing can serve as an important adjunctive tool to culture by providing a definitive etiology for intracerebral abscesses with negative culture results, allowing for more targeted treatment.

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**References**